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(54) Title: COMPOSITION FOR ORGAN CRYOPRESERVATION AND TREATMENT OF VIRAL AND BACTERIAL INFECTIONS

(57) Abstract

The invention provides a method for the cryopreservation of organs and the treatment of viral and/or microbial infections, the substance or composition comprising an active agent selected from the group consisting of amides of the general formula R_3-CO-NR_1R_2, in which: R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and R_3 is selected from H, Me and saturated an unsaturated C_2-C_3 alkyl groups.

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COMPOSITION FOR ORGAN CRYOPRESERVATION AND TREATMENT OF VIRAL AND BACTERIAL INFECTIONS

The invention relates, in particular, to a substance or composition for the cryopreservation of organs and the treatment of viral and/or microbial infections, to a substance or composition for the cryopreservation of organs, to a substance or composition for the treatment of viral and/or microbial infections, to a method of making a cryoprotective agent for the cryopreservation of organs, to a method of making a medicament or preparation for the treatment of viral and/or microbial infections, to the use in the cryopreservation of organs of a cryoprotective agent, to the use in the treatment of viral and/or microbial infections of a medicament or preparation, to the use of a cryoprotective agent in the manufacture of a cryopreservation agent, to the use of a substance or composition in the manufacture of a medicament or preparation for the treatment of a viral and/or microbial infection, to a method of cryogenically preserving an organ, to a method of thawing an

organ, to a method of treating a viral and/or microbial infection, to a dosage form and to a vaccine.

According to one aspect of the invention, there is provided a substance or composition for the cryopreservation of organs and the treatment of a viral and/or microbial infection, the substance or composition comprising an active agent selected from the group consisting of amides of the general formula R_3-CO-NR_1R_2, in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups.

At least one of R_1 and R_2 may be a methyl group.

Instead, at least one of R_1 and R_2 may be a fluorinated C_1 - C_3 alkyl group.

The active agent may be selected from the group including formamide, methyl formamide, dimethylformamide, acetamide,

methylacetamide, dimethylacetamide, diethylacetamide, isopropylacetamide, diisopropylacetamide, N-acetylpiperidine, N-(β-hydroxyethyl) acetamide, N,N-di(β-hydroxyethyl) acetamide, N-acetylmorpholine, acrylamide, propionamide, N-fluoromethyl-N-methyl-formamide and mixtures of any two or more thereof.

In one particular embodiment, the active agent may be dimethylformamide C_3H_7NO (DMF). In another particular embodiment the active agent may be N-fluoromethyl-N-methyl-formamide, $HCON(CH_3)(CH_2F)$.

DMF is generally used as a polar solvent and is readily absorbed through the skin, through the lungs or after oral exposure. The absorption rate of liquid DMF through the skin amounts to 9.4 mg per cm^2 per hour. DMF is rapidly metabolized, the main biotransformation site is the liver and excretion occurs for the larger part via the urine. The main metabolites in rat, mice, hamster and man are N-(hydroxymethyl)-N-methylformamide (HMMF), N-(hydroxymethyl)-formamide (HMF) and N-acetyl-S-(N-methyl-carbamoyl)cysteine (AMCC). Unchanged DMF is excreted in the urine as a small fraction of the dose. The limited data available indicate that a significant amount of the dose remains unexcreted and/or is excreted as unidentified compounds.

DMF has low acute dermal, oral and inhalation toxicity. It is considered to be a mild to moderate skin and eye irritant and

readily permeates the skin. There is no indication of skin sensitizing properties.

A NOAEL (No-Adverse-Observed-Effect-Level) could not be established in several 90-day studies. In a 28 day inhalation study with dogs, no effects were found at 63 mg/m^3. In another study with dogs, reversible cardiovascular effects were found at 60 mg/m^3.

DMF is teratogenic and probably embryolethal. The NOAEL for developmental effects amounted to 44 mg/kg body weight in an oral study and 150 mg/m^3 in an inhalation study with rabbits.

It is a particular feature of the invention that the toxicity of DMF during cryogenic procedures is substantially reduced or essentially eliminated by pre-cooling the organ before exposing it to DMF. By way of example, protein is denatured in a 30% DMF solution in water or a physiological solution at room temperature. However, at 8°C, in accordance with the method of the invention, an organ is essentially unaffected at a DMF concentration level of 30% and at a temperature of below 0°C, the organ is unaffected by a DMF concentration of 40%.

As is described in the Example below, an organ is generally unaffected during cryogenic procedures if a 10% solution of DMF is introduced at a temperature of between 8 - 4°C and if a 25% -

30% solution of DMF is introduced, the temperature of the organ and solution should be below 4°C.

During cryopreservation procedures, the substance or composition of the invention functions simultaneously as a cryopreservation agent and as a virostatic and/or bacteriostatic agent. Thus, if an organ is infected by a virus or by bacteria, cryopreservation of the organ will simultaneously destroy the viral and/or microbial infection.

For the purposes of this specification the word "organ" should be broadly construed to include within its scope heart, liver, skin, tissue, cornea, bone, glands and heart valves.

The invention provides a cryoprotective agent (CPA) and a method by which, for example, mammalian or other tissue, organs or entire bodies can be treated with the CPA, deep frozen and stored essentially indefinitely to be thawed when required. Generally, non-frozen donor organs have a shelf life of about 4 hours after which cell damage sets in thus making trans-continental shipment of donor organs difficult or impossible. The procedure of the invention, as will become evident below, is not complicated and the organ readily withstands temperature cooling rates of up to -10°C/second by immersion in liquid nitrogen after CPA treatment. Transcontinental shipment of donor organs and the establishment of donor organ banks or the

preservation of cadavers, limbs and organs for medical use thus becomes viable.

Thus, according to another aspect of the invention, there is provided a substance or composition for the cryopreservation of organs, the substance or composition comprising a cryogenic agent selected from the group consisting of amides of the general formula R_3-CO-NR_1R_2, in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups.

At least one of R_1 and R_2 may be a methyl group.

Instead, at least one of R_1 and R_2 may be a fluorinated C_1 - C_3 alkyl group.

The amide may be as hereinbefore described.

In one particular embodiment, the cryogenic agent may be dimethyl formamide. In another particular embodiment, the cryogenic agent may be N-fluoromethyl-N-methyl-formamide.

The cryogenic agent may be combined with a physiological solution comprising a component selected from the group consisting of sodium chloride, disodium-EDTA, sodium bicarbonate, potassium chloride, potassium dihydrogen phosphate, magnesium chloride, magnesium sulphate, calcium chloride, glucose, Hespes-sodium hydroxide and mixtures of any two or more thereof.

In particular, the physiological solution may be Tyrode's solution (referred to hereinafter as Tyrode), the University of Wisconson solution (ViaSpan), Krebs Henseleit or any other suitable physiological solution.

The invention extends to a substance or composition for the treatment of viral and/or microbial infections, the substance or composition comprising an active therapeutic agent selected from the group consisting of amides of the general formula R_3-CO-NR_1R_2, in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups.

R_1, R_2 and R_3 may be as hereinbefore described.

The amide may be as hereinbefore described.

In one particular embodiment, the amide may be dimethyl formamide. In another particular embodiment, the amide may be N-fluoromethyl-N-methyl-formamide.

The viral infection may be a retrograde viral infection eg an acquired human immunodeficiency (HIV-1) viral infection. The compound, substance or composition of the invention may, however, be used for the treatment of other viral and/or microbial infections such as German measles, skin acne and opportunistic infections associated with diseases of the immune system such as HIV-1. In particular, the substance or composition of the invention may be used for the treatment of viral infections in which the virus has a capsid protective coating.

Thus, the viral infection may be selected from acquired human immunodeficiency viral infections (HIV-1).

The substance or composition may include at least one physiologically acceptable excipient or carrier. The excipient or carrier may be colloidal silicon dioxide.

According to another aspect of the invention, there is provided a method of making a cryoprotective agent for the cryopreservation of organs, the method including the step of combining a cryoprotective component selected from the group which includes amides of the general formula R_3-CO-NR_1R_2, in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups

with a physiological solution.

The physiological solution may be as hereinbefore described.

R_1, R_2 and R_3 may be as hereinbefore described.

The amide may be as hereinbefore described.

According to another aspect of the invention, there is provided a method of making a medicament or preparation for the treatment of viral and/or microbial infections, the method including the step of combining an active therapeutic agent selected from the group which includes amides of the general formula R_3-CO-NR_1R_2, in which

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups,

with at least one physiologically acceptable excipient or carrier.

R_1, R_2 and R_3 may be as hereinbefore described.

The amide may be as hereinbefore described.

The invention extends to the use, in the cryopreservation of organs, of a cryoprotective agent comprising a cryoprotective component selected from the group which includes amides of the general formula R_3-CO-NR_1R_2, in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups.

The cryoprotective agent may comprise the cryoprotective component and a physiological solution.

R_1, R_2 and R_3 may be as hereinbefore described.

The amide may be as hereinbefore described.

The invention extends further to the use, in the treatment of viral and/or microbial infections, of a medicament or preparation comprising an active therapeutic agent selected from the group which includes amides of the general formula R_3-CO-NR_1R_2, in which

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups.

R_1, R_2 and R_3 may be as hereinbefore described.

The medicament or preparation may include at least one physiologically acceptable excipient or carrier.

The physiologically acceptable excipient or carrier may be colloidal silicon dioxide.

The amide may be as hereinbefore described.

The invention extends to the use of a cryoprotective component in the manufacture of a cryoprotective agent, the component being selected from the group consisting of amides of the general formula R_3-CO-NR_1R_2 in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups.

R_1, R_2 and R_3 may be as hereinbefore described.

The amide may be as hereinbefore described.

The invention extends, further, to the use of a substance or composition in the manufacture of a medicament or preparation for the treatment of a viral and/or microbial infection, the substance or composition comprising an active therapeutic agent selected from the group which includes amides of the general formula R_3-CO-NR_1R_2, in which

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups.

R_1, R_2 and R_3 may be as hereinbefore described.

The amide may be as hereinbefore described.

The invention extends, further, to a method of cryogenically preserving an organ, the method including the steps of perfusing the organ with a cryoprotective agent which includes a cryoprotective component selected from the group consisting of amides of the general formula R_3-CO-NR_1R_2 in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups and

reducing the temperature of the perfused organ to a preservation temperature at which the organ will, at least temporarily, be preserved.

R_1, R_2 and R_3 may be as hereinbefore described.

The amide may be as hereinbefore described.

Typically, the method will include initially perfusing the organ with a physiological solution such as Tyrode [TRADE NAME] and then, in a stepwise or continuous fashion, perfusing the organ with a physiological solution containing progressively

higher concentrations of the cryoprotective component whilst at the same time reducing the temperature of the organ.

Temperature reduction may be by initially cooling the organ and then rapidly freezing the organ in liquid nitrogen and storing the organ at liquid nitrogen temperature; by freezing the organ in liquid nitrogen and storing it in a freezer; by planner freezing and storage in liquid nitrogen; or by planner freezing and storage in a freezer.

The method may thus include the prior step of perfusing the organ with a physiological solution and cooling the organ to a temperature of between 8 to 5°C and then perfusing the organ with the cryoprotective agent whilst progressively increasing the concentration of the cryoprotective component in the cryoprotective agent and cooling the organ to the preservation temperature.

The concentration of the cryoprotective component may be progressively increased from 10% to 40%.

The preservation temperature will typically be a subzero temperature ranging from about -50°C to -196°C (liquid nitrogen temperature)

In particular, the preservation temperature may be between about -80°C and -196°C. This may be achieved using liquid CFC (chlorofluorocarbon) or a liquid nitrogen refrigerant.

The invention extends to a method of thawing an organ which has been perfused with a cryoprotective agent and cooled to a preservation temperature, the method including the steps of

exposing the organ to a cryoprotective agent which includes a cryoprotective component selected from the group consisting of amides of the general formula R_3-CO-NR_1R_2 in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups,

until the organ has reached a pre-determined temperature above the preservation temperature;

perfusing the organ with a cryoprotective agent containing the cryoprotective component while progressively reducing the concentration of the cryoprotective component in the cryoprotective agent and increasing the temperature of the organ; and

perfusing the organ with a physiological solution.

The pre-determined temperature may be about -4°C to 4°C.

R_1, R_2 and R_3 may be as hereinbefore described.

The amide may be as hereinbefore described.

The invention extends, still further, to a method of treating a viral and/or a microbial infection, the method including the step of administering to a subject having a viral and/or a microbial infection, a medicament or preparation comprising a physiologically effective dosage of an active therapeutic agent selected from the group which includes amides of the general formula R_3-CO-NR_1R_2, in which

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups.

The medicament or preparation may include at least one physiologically acceptable excipient or carrier as hereinbefore described.

R_1, R_2 and R_3 may be as hereinbefore described.

The amide may be as hereinbefore described.

The dosage may be selected so as to provide a blood concentration of the active therapeutic agent, in the blood of the subject being treated, of about 2 - 200 ppm, preferably about 50 - 100 ppm. A DMF plasma peak concentration of 50 - 100 ppm has been found to increase the CD4 T-cell count of a patient with HIV-1 from 312 to over 1000/mℓ within 3 weeks of commencing treatment.

The dosage may be administered transcutaneously, i.e. by means of skin transfer of the active therapeutic agent, eg through the application of a skin patch or a cotton wool pad which has been impregnated with the active therapeutic agent or with a composition comprising the active therapeutic agent. The concentration of the active therapeutic agent in the composition used to impregnate the skin patch or cotton wool pad may be about 10 - 100%.

The dosage may, instead, be administered by a method selected from oral dosages, inhalation, by means of a suppository, intravenously and combinations of any two or more thereof. The intravenous introduction will typically be at a controlled rate over a period of time.

The invention thus extends to a dosage form for the treatment of a viral and/or microbial infection which comprises topical application means, such as a skin patch or pad, on which is adsorbed or absorbed an effective amount of an active therapeutic agent as hereinbefore described.

The topical application means may comprise a body of a synthetic polymeric material such as TEFLON (trade name) on which the active therapeutic agent is adsorbed or absorbed. Typically, the dosage form will include DMF admixed with colloidal silicon dioxide to form a gel with which the patch is impregnated. The patch may be a Virodene P058 patch.

The invention extends to a vaccine prepared from antibodies harvested from the body of a person, after the person has been infected with a virus and who has then been treated in accordance with the method of the invention.

The virus may be a retrograde virus such as HIV-1.

The invention is now described, by way of example, with reference to the accompanying Figures and Examples, in which

Figure 1 shows temperature changes at the aortic cannula during immersion of rat heart in liquid nitrogen;

Figure 2 is a schematic diagram showing a cannulation procedure and position of thermocouples in the heart;

Figure 3 shows HIV-1 viral load (polymerase chain reaction [PCR]) as a function of time for an aids patient treated with Virodene PO58 (DMF) patches;

Figure 4 shows temperature as a function of time for entire rat heart profusion, cryopreservation, freezing, thawing and re-perfusion in accordance with the method of the invention;

Figure 5 shows temperature as a function of time for thermocouple calibration and transient response;

Figure 6 shows plasma DMF concentrations as a function of time during a first set of human toxicity trials;

Figure 7 shows plasma DMF concentrations as a function of time during a second set of human toxicity trials;

Figure 8 is a micrograph of a subendocardium of a frozen/thawed rat heart after a cryopreservation process in accordance with the method of the invention at 22500 times enlargement;

Figure 9 is a micrograph of a subendocardium of a frozen/thawed rat heart after a cryopreservation process in accordance with the method of the invention at 36250 times enlargement; and

Figure 10 is a micrograph of a subendocardium of a frozen/thawed rat heart after a cryopreservation process in accordance with the method of the invention at 28500 times enlargement.

EXAMPLE 1

CRYOPRESERVATION

MATERIALS and METHODS

Model: Isolated perfused rat heart

Twelve BD9 rats, each weighing 250-350g were used. The rats were anaesthetised with sodium pentobarbitone according to an accepted protocol.

Langendorff Apparatus

Two standard Langendorff systems were used, connected to two water baths, one was a low temperature both for cooling the organ to approximately 4-5 °C and the other was maintained at 37 °C. The cold Langendorff system (referred to as LD2) was used to perfuse the heart with a Tyrode-DMF-solution, and the warm Langendorff system (referred to as LD1) to perfuse the heart in the absence of DMF, ie with pure Tyrode.

Perfusate composition per litre of solution (Tyrode)

NaCl	137.6 mM
KCl	5.4 mM
$MgCl_2$	1.0 mM
$CaCl_2$	1.8 mM

Glucose	5.0 mM
Hespes-NaOH	11.6 mM
pH of solution	7.45

DMF Properties (BASF Industrial Chemicals, Dimethylformamide Technical Leaflet M5351e, October, 1989)

N,N-Dimethylformamide (DMF) is a cell differentiator with the following properties

Molecular formula:	C_3H_7ON
Relative molar mass:	73,095 g/mol
Freezing point:	-61°C

DMF is a colourless, high boiling point, mobile, polar, hygroscopic liquid with a faint characteristic odour. As an aprotic solvent with a high dielectric constant, DMF is an excellent solvent, is both water and lipid soluble and has a low vapour pressure at 23°C. DMF in contact with strong oxidizers, alkylaluminium and halogenated chemicals can result in fires or explosions.

DMF was stored in an airtight container below - 5 °C. Perfusate / CPA (OV) solutions were mixed in quantities as and when required and were not stored for later use.

Temperature measurement

Temperature was continually measured by means of a small (0.5 mm diameter) T-type thermocouple positioned inside the aortic cannula as shown schematically in Figure 2. This position was chosen as the best compromise between proximity to the heart and minimum interference with the movement of the heart. The thermocouple was accurately able to follow temperature changes at a rate in excess of 45 °C/s. The temperature was sampled once per second throughout the experiment using a PC-30 12 bit A/D card (Eagle Electronics) with a custom-built amplifier incorporating cold point temperature compensation. The temperature measuring system was calibrated against a commercial Fluke 52 K-type thermocouple thermometer. Measurements over the range - 100°C to + 100°C were verified to be accurate to within 2% of the true temperature. During the rapid cooling phase in liquid nitrogen, the heart was submerged in liquid nitrogen to just above the aorta (see Figure 2). Temperature measurements during the cryopreservation experiments were taken inside the left ventricle as well as just inside the aortic cannula as mentioned above. An intracardiac temperature of -196°C was reached within 20 seconds of submerging the heart in liquid nitrogen, correlated to -75°C at the tip of the aortic cannula. The cooling rate was approximately 10°C/s during the rapid cooling phase in the left ventricle and 7,5°C/s at the tip of the aorta.

Perfusate / CPA (OV) mixtures used:

Tyrode 90% and DMF 10% referred to as (OV1) solution
Tyrode 80% and DMF 20% referred to as (OV2) solution
Tyrode 75% and DMF 25% referred to as (OV25) solution
Tyrode 70% and DMF 30% referred to as (OV3) solution
All percentages refer to volume/volume (v/v) percentages

Technique

A warm/cold (37°C/5°C) LD1 and a cold (5°C) LD2 Langendorff system as described above were prepared. In LD1 only pure Tyrode solution was used and the system was used to cool all the perfused rat hearts. In LD2 both pure tyrode and OV mixtures of cyroprotectant were used only at the cold (5°C) temperature.

The DMF was kept in a cold storage area at -5°C in an airtight container. Solutions of 10%, 20%, 25% and 30% DMF in Tyrode solution were made up immediately before use.

In a first set of experiments, the rats were anaesthetized and the heart excised, arrested in cold Tyrode solution, cannulated through the aorta, and connected to the warm/cold Langendorff system (LD1) containing pure Tyrode solution at 37°C and perfused for eight minutes to stabilise contractions (Figure 4 - annotation a) after which the system temperature was dropped

to 5°C and maintained at this temperature for 10 minutes (Figure 4 - annotations b - c).

The excision was carried out by cutting the abdomen transversely just below the rib cage with Mayo scissors, exposing the diaphragm and then cutting the diaphragm away around the ventral and lateral margins to expose the heart and lungs. At this point, the lungs no longer function and the ischemic clock starts running. The mediastinum was then cut away between the heart and rib cage and the heart was gently lifted and cut away with iris scissors. The heart was then dropped into a beaker of cold Tyrode to arrest it. The heart was then lifted by the cut edges of the aorta and the aorta was secured to the cannula by means of cotton thread and the ends of the suture were trimmed.

The heart was then disconnected from the LD1 system and connected to the LD2 system (Figure 4 - annotations c - d). The perfusion of the heart from the LD2 system was started with pure Tyrode at 5°C and the perfusion solution was then changed to 50mℓ OV1 solution (LD2) (Figure 4 - annotation d). The heart was perfused for ten minutes until the OV1 solution was depleted. System LD2 was then filled with 30mℓ of OV2 solution and perfusion was continued for eight minutes. System LD2 was then filled with 30mℓ of OV3 solution and, after eight minutes just before the OV3 was depleted, perfusion was stopped. The heart was then covered with a thin layer of cotton wool, soaked with OV3 solution at 0°C. The heart was then submerged completely in

liquid nitrogen, until the temperature of the left ventricle reached -196°C. This took about 20 seconds at a cooling rate of -10°C/second. The heart was maintained at a temperature of -196°C for periods ranging from 10 seconds up to 45 minutes before rewarming.

The heart was then placed in a 100 mℓ glass beaker containing 70 mℓ of OV3 at a temperature of 0°C and left to thaw for twelve minutes (Figure 4 annotations g - i). After this time the left ventricle temperature was between +4°C and -4°C. Perfusion was restarted on the cold (5°C) still connected Langendorff system (LD2) using 20mℓ of OV3 for 2 minutes. The LD2 system was then filled with 20mℓ of OV2 solution and perfusion was continued for 2 minutes, then with 30mℓ of OV1 solution and perfusion was continued for four minutes, and then with 100mℓ of pure Tyrode and perfusion was continued for twelve minutes. Total reperfusion time was 20 minutes. The heart was then removed and reconnected to the cold/warm Langendorff system LD1 (Figure 4 - annotations j - k), and perfused with pure Tyrode solution starting at a temperature of 5°C (Figure 4 - annotation k) and the system was reheated to a temperature of 37°C (Figure 4 - annotations k - m), until the left ventricle temperature was 37°C. The heart started contracting within 3 to 6 minutes upon reaching a temperature of between 14 - 30 °C. The beating heart was kept on the warm Langendorff system for 30 minutes, after which the experiment was terminated.

In a second set of experiments, OV1 and OV25 solutions were used. In this set of experiments, OV25 is substituted for OV2 and OV3.

If cooling in liquid nitrogen is initiated above 8°C, cracking of the heart generally occurs. The cottonwool wrapping slows the temperature drop by forming an insulating capsule around the heart.

All solutions were oxygenated by pre-bubbling with a 95% oxygen/5% CO_2 gas mixture. Diffusion of DMF into the cells was sufficiently fast that osmotic swelling was not observed. Bubbles in the perfusate entering the heart must be avoided as they result in infarcts which cause ischemia and interfere with the addition and removal of CPA. Large bubbles are lethal. The pressure head on the perfusate was preferably about 90 - 100 cm. A pressure head of about 37,5 cm resulted in inadequate perfusion.

Transmission electron microscopy of the sub-endocardial and sub-epicardial layers was done on all the hearts at completion of the experiment (see Figures 8 - 10).

The experiments described above were repeated on over 100 rat hearts.

RESULTS

Rapid cooling was achieved with temperature cooling rates of -75°C/s as can be seen in Figure 1 - annotation b. The temperature at the aortic cannula tip stabilised at -75°C with the heart submerged in liquid nitrogen. This correlates to an intracardiac temperature of -196°C. Rewarming, after the heart was removed from the liquid nitrogen was also rapid up to -7°C (see Figure I - annotation c)

The hearts in this series of experiments started contracting three to six minutes after warm perfusion on the Langendorff system was restarted, at myocardial temperatures ranging from 14 - 30°C. The active, rhythmically beating heart, was observed for at least 30 minutes. Coronary flow returned to pre-freeze rates and the heart rate recovered to pre-freeze values. Macroscopically no damage could be seen. Electron microscopy showed no discernible damage, to the intracellular structures at magnifications of 22500 times, at 28500 times and at 36250 times (see Figures 8 - 10).

In the case of the cryopreservation of a liver, it is important to arrest liver function before the CPA is introduced, as a functional liver will break down DMF into toxic compounds (AMCC). DMF is hepatotoxic. Liver function ceases at about 8°C and a liver must be cooled to a minimum of 4°C before DMF is introduced. A complete body must be kept at or below 4°C before

introduction of DMF solutions. To freeze an entire body, the body is placed on a heart lung machine and the blood is replaced by a pure physiological solution and cooled to 4°C. CPA (DV solutions) are introduced and circulated through the body until all cells are saturated with CPA. The body is then covered in wadding soaked with CPA solution and frozen in liquid nitrogen. On reheating, a minimum temperature of 4°C must be maintained until all CPA solution has been flushed from the body cells. The body is then re-heated with pure physiological solution until 11°C whereafter blood is re-introduced into the body.

DISCUSSION

Previous attempts to preserve solid organs by freezing at temperatures approaching - 196°C whilst regaining functional status after the freezing process have been unsuccessful (Wang T, Banker MC, Clayden N, Hicks GL Jr, Layne JR Jr, Freezing preservation of the mammalian cardiac explant VI. Effect of thawing rate on functional recovery, Cryobiology 29, (470-477), 1992; Wang T, Connery CP, Batty PR, Freezing preservation of adult mammalian heart at high sub zero temperatures, Cryobiology, 29 (171-176), 1991; Wicomb WN, Hill DJ, Collins GM, Twenty four hour ice storage of rabbit heart, Journal of Heart and Lung Transplantation, 13:5, (891-894), 1994). As far as the Applicant is aware, there have also not been any successful experiments in which solid organs were frozen at temperatures below -80°C, and

subsequently showed no discernible damage to the organ at macroscopic or microscopic evaluation.

Intracellular damage is caused by crystallisation, by toxicity and osmotic stress. These problems are well documented in cryo journals. Reported previous successes with cryopreservation were only with single cells and sperm. These processes generally used slow or planner freezing, and where the absolute volume of the structure is very small (Rall WF, Fahy GM, Ice-free cryopreservation of mouse embryos at - 196°C by vitrification, Nature, 313, (573-575), 1985). In single cells, high temperature cooling rates and freezing are presumed to have a smaller effect because volumetric changes have less damaging effects on single cells than on intact organs.

A 25% DMF and Tyrode solution did not express volumetric expansion, as did an electrolyte-water solution, when submerged in liquid nitrogen. Therefore this concentration was used by the inventor in some of the rat heart experiments.

The temperature cooling rates encountered in the rat heart experiments did not have any deleterious effects, and no intracellular damage occurred

For successful cryopreservation of whole organs a medium and a process with the following properties is required:

1. The medium must rapidly displace intracellular water;
2. The medium must reach all the cells in the organs simultaneously (Burrows FA, Bissonette B, Cerebral Blood Flow Velocity Patterns During Cardiac Surgery Utilising Profound Hypothermia With Low Flow Cardiopulmonary Bypass or Circulatory Arrest in Neonates and Infants, Can Anaesth, 40 : 4, (298-307), 1993);
3. The medium must alter the properties of the mixture of water plus medium so that no or little volumetric changes take place upon freezing;
4. The medium must be non-toxic to cells and tissue;
5. The medium characteristics must be maintained over a broad temperature rang;
6. The medium must have a low viscosity at freezing temperatures, typically close to that of water;
7. The medium must be in a liquid state at sub-zero temperatures.

Dimethyl formamide (DMF) was found by the inventor to comply with most of the abovementioned requirements.

The rat heart was used as a model because it readily lends itself to evaluation in experimental work. The manner in which function can be tested in the rat heart in experimental models is well established, and comparative information is therefore available. Interest was primarily focused on the function of the heart after it had been subjected to cryopreservation techniques.

However, electron-microscopy of the tissue was conducted in all cases to investigate the possibility of ultra-structural changes.

The electron microscopy showed no discernible damage to the intracellular structures despite a very meticulous search for signs of cellular damage.

The hearts in the experiment recovered remarkably well after being subjected to extreme temperature cooling rates as well as very low endpoint temperatures. Macroscopically no damage could be seen, and the functionality was excellent as inferred from the motion of the heart contractions.

The invention opens the potential for long term storage of solid organs and tissue structures and the establishment of organ banks.

EXAMPLE 2

TREATMENT OF HIV

Toxicity studies were conducted on humans as were stage 3 clinical trials on HIV-1 positive patients using Virodene P058 skin patches. In vitro studies of DMF on cell cultures were also conducted by the inventor.

A patient infected with HIV-1 was treated with dimethylformamide (Virodene P058) by the application of skin patches impregnated with a dimethylformamide gel to the patient's body. Two skin patches were applied to different parts of the patient's body, for example to the forearm. Each skin patch contained about 7,064g of a gel comprising DMF (92,5 % m/m) and colloidal silicon dioxide (7,5 % m/m). The gel served to prevent leakage of liquid DMF from the patches. The patches were manufactured at most 12 hours prior to use as DMF evaporates rapidly. The intended level of DMF in the patient's blood is 100 ppm. For a patient weighing about 60 kg, an amount of about 14 g over a period of 12 hours is required to produce a level of 100 ppm. N-acetyl-cysteine-glutathion and/or essential phospholipids were administered (orally or intravenously) to the patient at a dosage of 250 mg to 300 mg daily as a liver booster. Instead or in addition, glutamine may also be administered to the patient as a liver booster. Based on studies by Marz and Nohova the surface area required to absorb this amount is about 127,2 cm^2. To obtain a blood level of 100 ppm about 1,272 g of DMF must be

absorbed per hour, thus each sticker requires about 7,064 g DMF to deliver the required amount, having a surface area of 6,36 cm^2 (each sticker). DMF absorption rate is 9.4 mg/cm^2/hour. In theory this treatment will deliver 125 - 135 ppm, but due to evaporation of the DMF, 100 ppm is obtained. Absorption capability varies from patient to patient depending on factors such as skin-type and skin thickness. To obtain the desired levels of DMF in the patients, plasma DMF concentrations were monitored for each patient and treatment adjusted accordingly depending on the DMF level of each patient (see Figure 6 and 7 for examples of various plasma DMF concentration levels in patients receiving the same treatment).

The stickers were thus each loaded with about 7,064 g of the gel of DMF and silicon dioxide. Each patch was applied for a period of 12 hours, either once per week over a period of 12 weeks, or twice per week over a period of 6 weeks.

Blood tests in certain patients indicated an increase in CD4 T-cell counts from 350 to 1000, and a rapid reduction of PCR (Polymerase chain reaction) (viral load) of 120000 to 500/mℓ within three weeks (see Figure 3) with as little as three treatments. The PCR test conducted is the Roche amplicor HIV monitor. A viral load of < 500/mℓ plasma is considered to be undetectable.

Some patients undergoing treatment had severe acne or displayed German measles symtoms before treatment. When treated with DMF so that the DMF level in the patient's blood was 50 - 100 ppm, the German measles symptoms and the severe acne cleared up or disappeared within 7 days.

Prior to the treatment with dimethylformamide, a comprehensive base-line clinical and psychological evaluation of the patient was conducted. The evaluation provided base-line biochemical and haematological data on the patient. Detailed virological serology (HIV-1) tests were also conducted to determine the patient's total body virus count, and these tests were conducted on a weekly basis, or as per treatment.

The concentration of DMF in the patient's blood was determined hourly during the period of treatment. An intravenous line was introduced each morning to take blood samples and was kept open with an infusion of Normal Saline at a rate of 20mℓ/h and daily monitoring of the active metabolite AMCC (eg by 4 hourly urine sampling) derived from the DMF was also conducted. Subsequent applications of DMF were adjusted in accordance with measured changes in blood level DMF concentration resulting from changes in absorbtion variables and daily full haematological and biochemical profiles were conducted to detect any changes in liver function. Daily full clinical and psychological evaluations were also conducted.

A daily virological serology workout to establish total body virus count and to monitor improvements in the immune status of the patient (CD4 T-helper cells) and prognostic factors were also conducted. The serology workout was based on p24 antigen and quantitative PCR or, optionally, by other methods. A weekly determination of CD4 counts and Beta-2-macroglobulin was also conducted specifically to monitor improvements in the patient's immune status and prognosis. All clinical and laboratory data was fed into a centralised data system to facilitate rapid response to any detrimental change so as to curtail treatment to maximise clinical effect and minimise potential side effects.

The tests included

a) Serum:- S-Na,S-K,S-Cl, S-CO_2,S-Urea,Surate, S-Creat,S-Ca,S-Ca,S-Mg,S-Phos, Serum Total, S-Conjd;

b) Haemoglobin:- HB Det, Red cells, Haematocrit, MCV, MCH, MCHC, RDW;

c) Protein Electrophoresis: ST-Protein,S-Albumin,S-Total Glob, S-Alphal Glob, S-Alpha2 Glob, S-Beta Glob, S-Gamma Glob;

d) White cell differential count:-Wh cell count, Neutro absolute,Lypho absolute, Mono absolute, Eosino absolute, Baso absolute;

e) Liver Enzymes:- S-Alk.Phos,S-Gamma GT, S-Alt)SGPT), S-AST(SGOT), S-LD;

f) Virology:- Cell markers, PCR, Beta2-Microglobulin,P24 Antigen, C-Reactive protein,& CK-MB concentration;

g) blood analysis for DMF levels;

h) urine analysis for AMCC levels

It appears as if DMF acts as at least one of a reverse transcriptase inhibitor and a protease inhibitor. In vitro tests were conducted and it appears that the solvent properties of DMF dissolves the virus particles, e.g. the capsid.

CLAIMS

1. A substance or composition for the cryopreservation of organs and the treatment of viral and/or microbial infections, the substance or composition comprising an active agent selected from the group consisting of amides of the general formula R_3-CO-NR_1R_2, in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups.

2. A substance or composition as claimed in Claim 1, in which at least one of R_1 and R_2 is a methyl group.

3. A substance or composition as claimed in Claim 1, in which at least one of R_1 and R_2 is a fluorinated C_1-C_3 alkyl group.

4. A substance or composition as claimed in Claim 3, in which the active agent is N-fluoromethyl-N-methyl-formamide.

5. A substance or composition as claimed in Claim 1 or Claim 2, in which the active agent is dimethyl formamide.

6. A substance or composition for the cryopreservation of organs, the substance or composition comprising an active cryogenic agent selected from the group consisting of amides of the general formula R_3-CO-NR_1R_2, in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups.

7. A substance or composition as claimed in Claim 6, in which at least one of R_1 and R_2 is a methyl group.

8. A substance or composition as claimed in Claim 6, in which at least one of R_1 and R_2 is a fluorinated C_1-C_3 alkyl group.

9. A substance or composition as claimed in Claim 8, in which the active cryogenic agent is N-fluoromethyl-N-methyl-formamide.

10. A substance or composition as claimed in Claim 6 or Claim 7, in which the active cryogenic agent is dimethyl formamide.

11. A substance or composition as claimed in any one of Claims 5 to 10 inclusive, in which the cryogenic agent is combined with a physiological solution comprising a component selected from the group consisting of sodium chloride, disodium-EDTA, sodium bicarbonate, potassium chloride, potassium dihydrogen phosphate, magnesium chloride, magnesium sulphate calcium chloride, glucose and Hespes-sodium hydroxide and mixtures of any two or more thereof.

12. A substance or composition for the treatment of viral and/or microbial infections, the substance or composition comprising an active therapeutic agent selected from the group consisting of amides of the general formula R_3-CO-NR_1R_2, in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups

13. A substance or composition as claimed in Claim 12, in which the viral infection is selected from acquired human immunodeficiency viral infections (HIV-1).

14. A substance or composition as claimed in Claim 12 or Claim 13, in which at least one of R_1 and R_2 is a methyl group.

15. A substance or composition as claimed in Claim 12 or Claim 13, in which at least one of R_1 and R_2 is a fluorinated C_1-C_3 alkyl group.

16. A substance or composition as claimed in Claim 15, in which the active therapeutic agent is N-fluormethyl-N-methyl-formamide.

17. A substance or composition as claimed in any one of Claims 12 to 14 inclusive, in which the active therapeutic agent is dimethyl formamide.

18. A substance or composition as claimed in any one of Claims 12 to 17 inclusive, which includes at least one physiologically acceptable excipient or carrier.

19. A substance or composition as claimed in Claim 18 in which the physiologically acceptable excipient or carrier is colloidal silicon dioxide.

20. A method of making a cryoprotective agent for the cryopreservation of organs, the method including the step of combining a cryoprotective component selected from the group which includes amides of the general formula R_3-CO-NR_1R_2 in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups

with a physiological solution.

21. A method of making a medicament or preparation for the treatment of viral and/or microbial infections, the method including the step of combining an active therapeutic agent selected from the group which includes amides of the general formula R_3-CO-NR_1R_2 in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups.

with at least one physiologically acceptable excipient or carrier.

22. Use, in the cryopreservation of organs of a cryoprotective agent comprising a cryoprotective component selected from the group which includes amides of the general formula R_3-CO-NR_1R_2, in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups.

23. Use, in the treatment of viral and/or microbial infections, of a medicament or preparation comprising an active therapeutic agent selected from the group which includes amides of the general formula R_3-CO-NR_1R_2, in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups.

24. Use of a cryoprotective component in the manufacture of a cryoprotective agent, the component being selected from the group consisting of amides of the general formula R_3-CO-NR_1R_2 in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups.

25. Use of a substance or composition in the manufacture of a medicament or preparation for the treatment of a viral and/or microbial infection, the substance or composition comprising an

active therapeutic agent selected from the group consisting of amides of the general formula R_3-CO-NR_1R_2 in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups.

26. A method of cryogenically preserving an organ, the method including the steps of perfusing the organ with a cryoprotective agent which includes a cryoprotective component selected from the group consisting of amides of the general formula R_3-CO-NR_1R_2 in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups and

reducing the temperature of the perfused organ to a preservation temperature at which the organ will, at least temporarily, be preserved.

27. A method as claimed in Claim 26, which includes the prior steps of perfusing the organ with a physiological solution and cooling the organ to a temperature of 5 to 8 °C and then perfusing the organ with the cryoprotective agent whilst progressively increasing the concentration of the cryoprotective component in the cryoprotective agent and cooling the organ to the preservation temperature.

28. A method as claimed in Claim 27, in which the concentration of the cryoprotective component is progressively increased to 25% - 40%.

29. A method as claimed in Claim 27 or Claim 28, in which the preservation temperature is between -4°C and -196°C.

30. A method of thawing an organ which has been perfused with a cryoprotective agent and cooled to a preservation temperature, the method including the steps of exposing the organ to a cryoprotective agent which includes a cryoprotective component selected from the group consisting of amides of the general formula R_3-CO-NR_1R_2 in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3

alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups

until the organ has reached a pre-determined temperature above the preservation temperature;

perfusing the organ with a cryoprotective agent containing the cryoprotective component while progressively reducing the concentration of the cryoprotective component in the cryoprotective agent and increasing the temperature of the organ; and

perfusing the organ with a physiological solution.

31. A method of treating a viral and/or microbial infection, the method including the step of administering to a subject having a viral and/or microbial infection, a medicament or preparation comprising a physiologically effective dosage of an active therapeutic agent selected from the group which includes amides of the general formula R_3-CO-NR_1R_2 in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups.

32. A method as claimed in Claim 31, in which the dosage is selected so as to provide a blood concentration of the active agent in the blood of the subject being treated of about 50 - 200 ppm.

33. A method as claimed in Claim 32, in which the dosage is selected so as to provide a blood concentration of 50 -500 ppm.

34. A method as claimed in any one of Claims 31 to 32 inclusive, in which the dosage is administered transcutaneously.

35. A method as claimed in any one of Claims 31 to 34 inclusive, in which the dosage is administered by a method selected from oral dosages, inhalation, by means of a suppository, intravenously and combinations of any two or more thereof.

36. A dosage form for the treatment of a viral and/or microbial infection which comprises topical application means on which is absorbed or adsorbed an effective amount of an active therapeutic agent selected from the group consisting of amides of the general formula R_3-CO-NR_1R_2, in which:

R_1 and R_2 are independently selected from the group including H, Me, halomethyl, saturated and unsaturated C_2-C_3 alkyl groups, saturated and unsaturated halogenated C_2-C_3 alkyl groups, hydroxylated alkyl groups; or

R_1 and R_2 are together selected from $(CH_2)n$, wherein n = 4 or 5, or $(CH_2)_2O(CH_2)_2$; and

R_3 is selected from H, Me and saturated and unsaturated C_2-C_3 alkyl groups.

37. A vaccine prepared from antibodies harvested from the body of a person after the person has been infected with a virus and has been treated in accordance with the method as claimed in any one of Claims 31 - 35 inclusive.

38. A substance or composition for the cryopreservation of organs and the treatment of viral and/or microbial infections substantially as hereinbefore described and illustrated.

39. A substance or composition for the cryopreservation of organs substantially as hereinbefore described and illustrated.

40. A substance or composition for the treatment of viral and/or microbial infections substantially as hereinbefore described and illustrated.

41. A method of making a cryoprotective agent substantially as hereinbefore described and illustrated.

42. A method of making a medicament or preparation substantially as hereinbefore described and illustrated.

43. Use, in the cryopreservation of organs of a cryoprotective agent substantially as hereinbefore described and illustrated.

44. Use, in the treatment of viral and/or microbial infections of a medicament or preparation substantially as hereinbefore described and illustrated.

45. Use of a cryoprotective component in the manufacture of a cryoprotective agent substantially as hereinbefore described and illustrated.

46. Use of a substance or composition in the manufacture of a medicament or preparation substantially as hereinbefore described and illustrated.

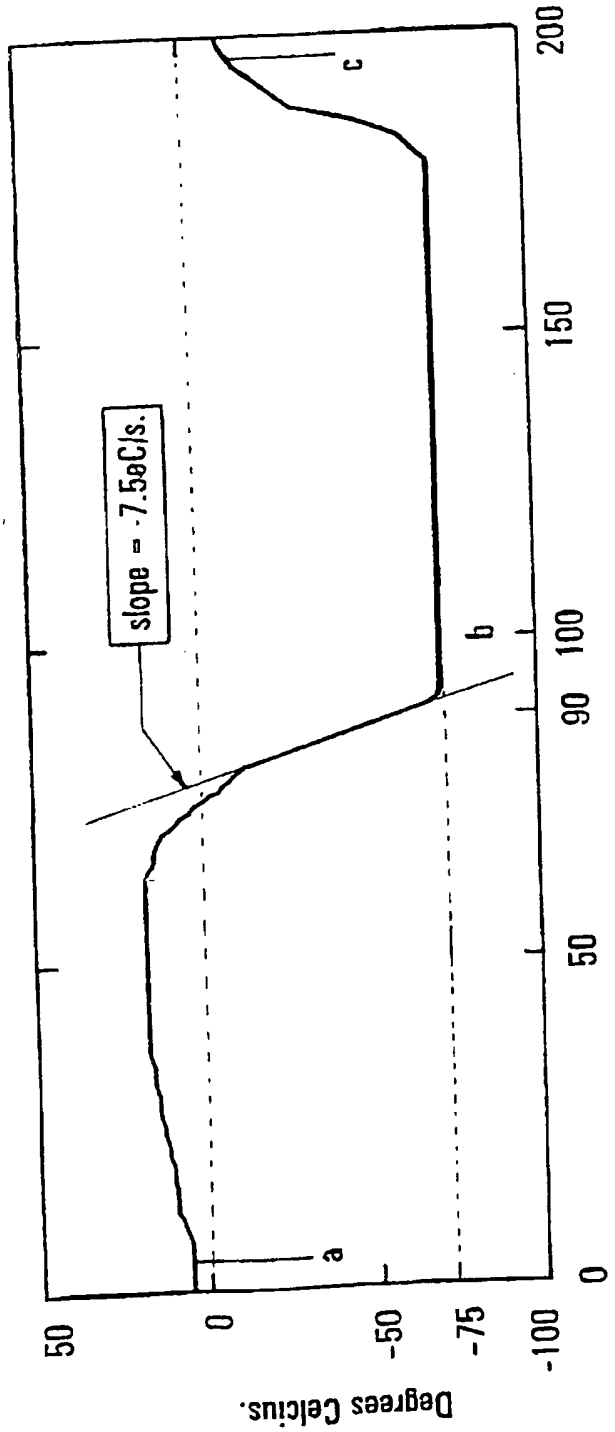
47. A method of cryogenically preserving an organ substantially as hereinbefore described and illustrated.

48. A method of thawing an organ substantially as hereinbefore described and illustrated.

49. A method of treating a viral and/or microbial infection substantially as hereinbefore described and illustrated.

50. A dosage form substantially as hereinbefore described and illustrated.

51. A vaccine substantially as hereinbefore described and illustrated.



Temperature changes at aortic cannula during immersion of rat heart in liquid nitrogen.

FIG 1

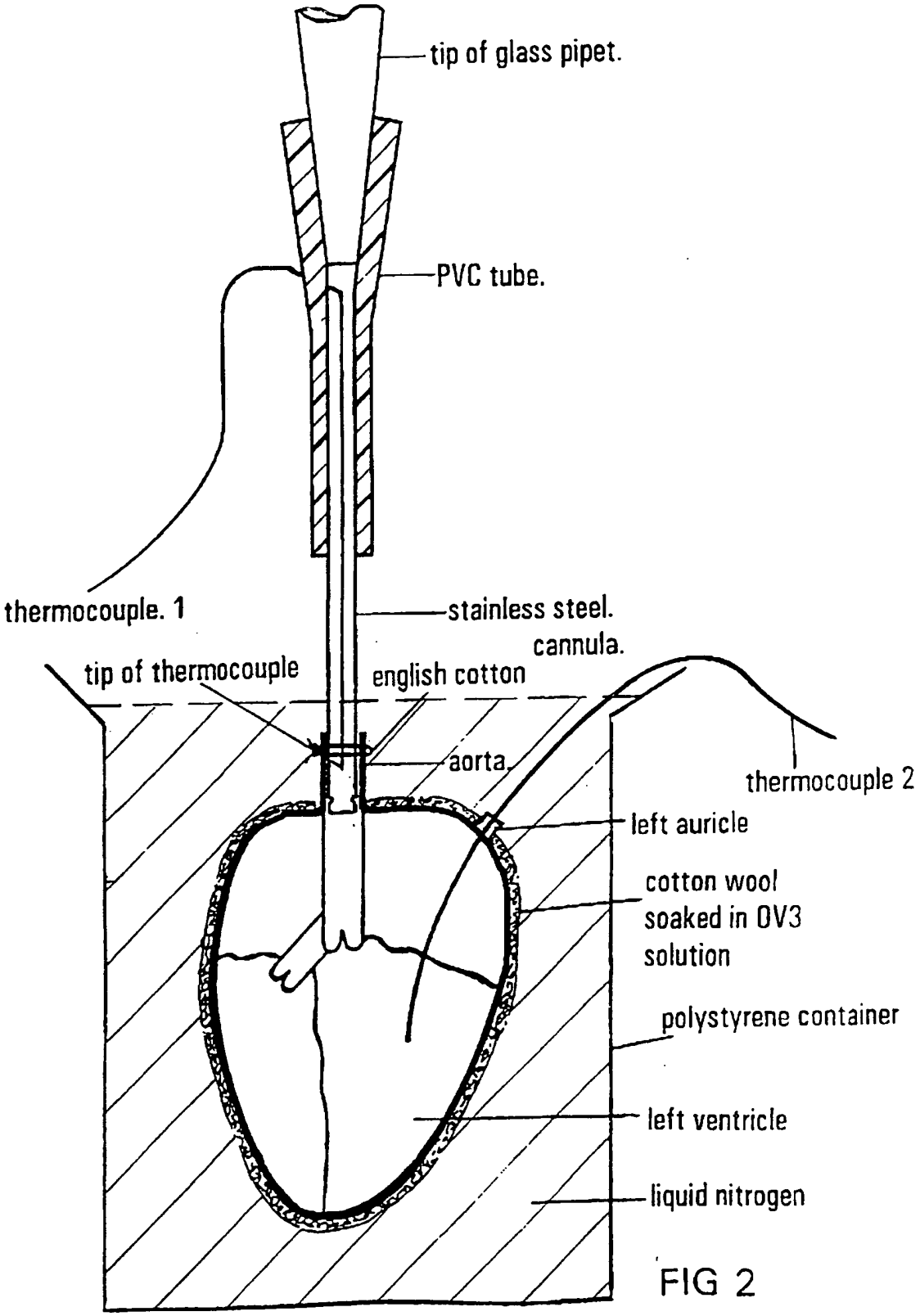

tip of glass pipet.
PVC tube.
thermocouple. 1
stainless steel.
cannula.
tip of thermocouple
english cotton
aorta
thermocouple 2
left auricle
cotton wool
soaked in OV3
solution
polystyrene container
left ventricle
liquid nitrogen

FIG 2

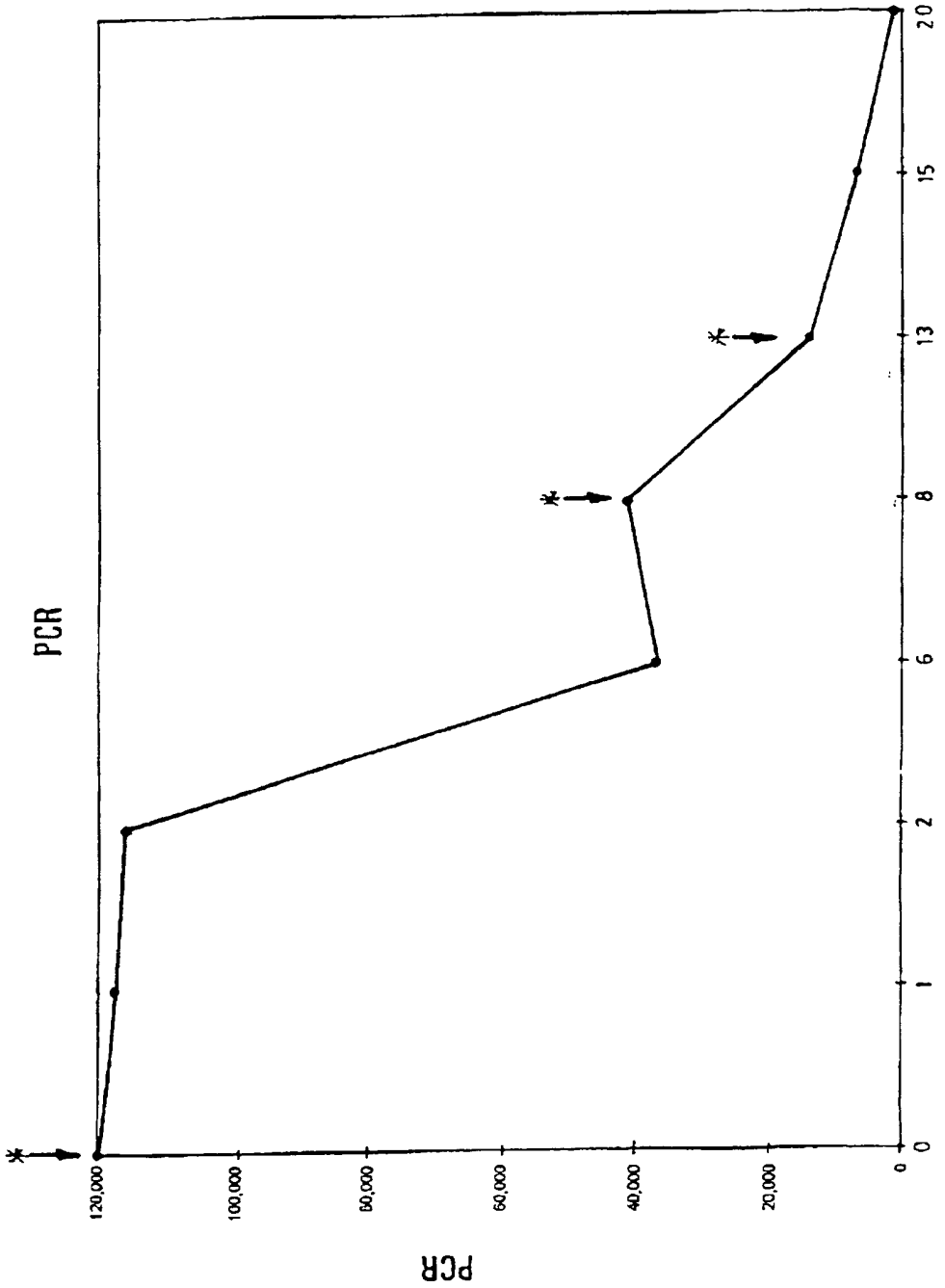

PCR
120,000
100,000
80,000
60,000
40,000
20,000
0
1
2
6
8
13
15
20
DAYS
* Treatment (2 Patches virodene P058 for 12 hours)

FIG 3

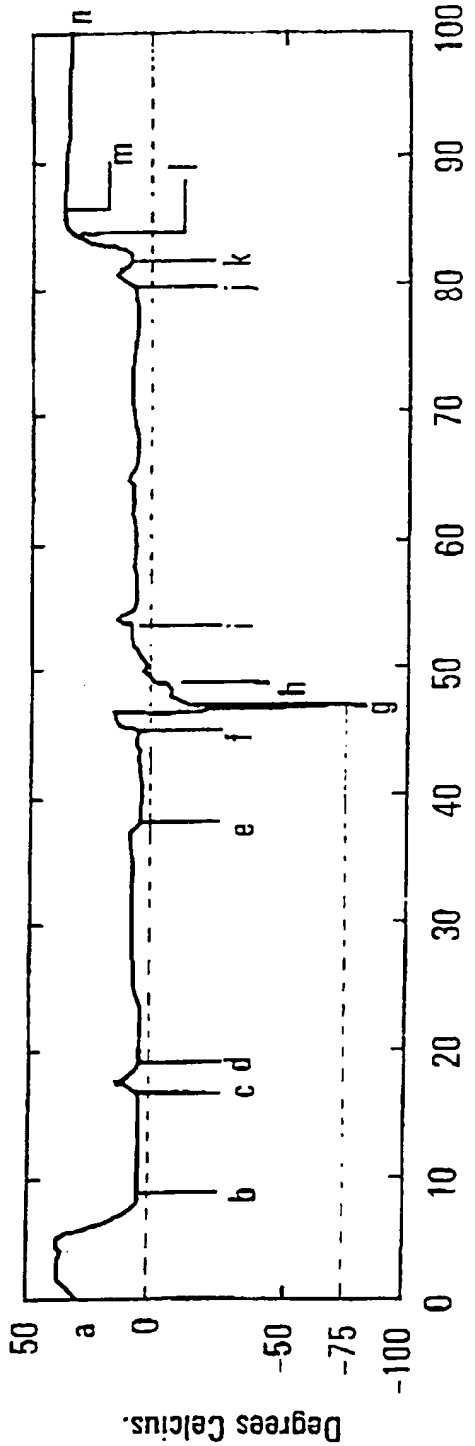

Degrees Celcius.
50
0
-50
-75
-100
0
10
20
30
40
50
60
70
80
90
100
Minutes.
a
b
c
d
e
f
g
h
i
j
k
l
m
n

FIG 4

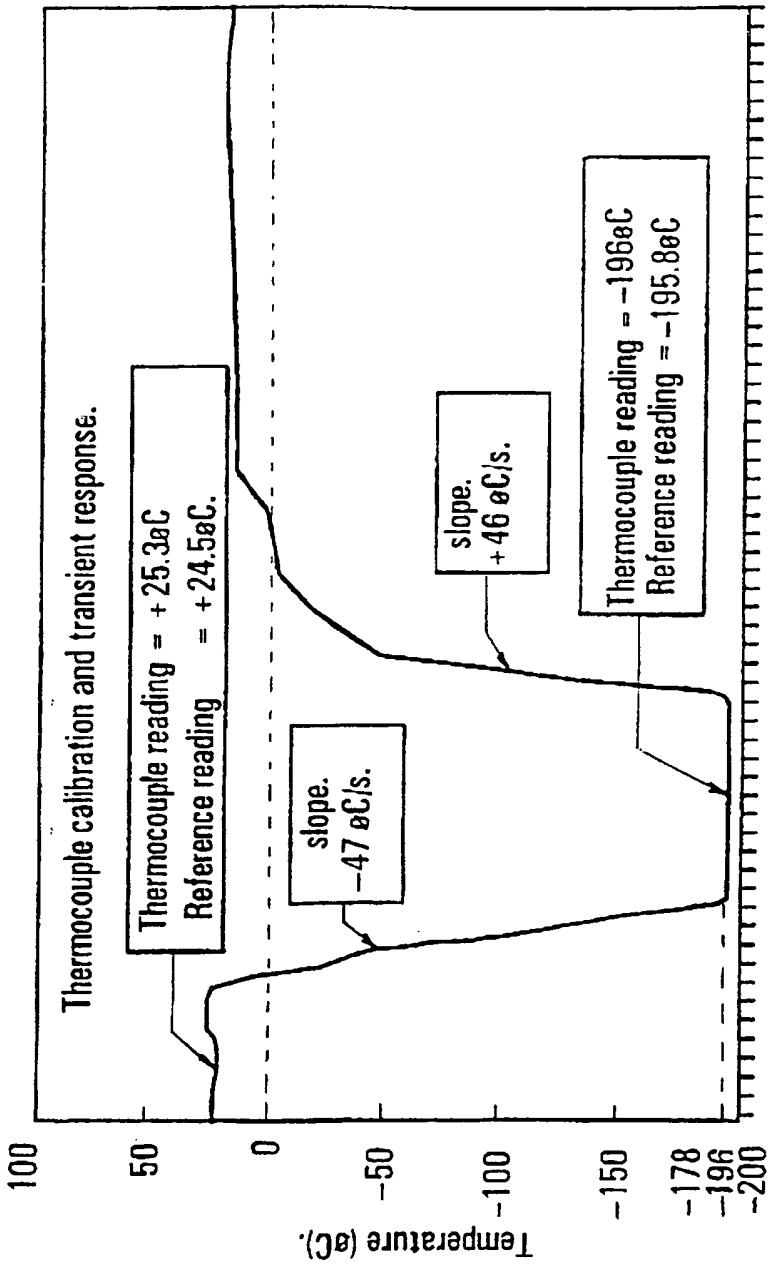

Thermocouple calibration and transient response.
Thermocouple reading = +25.3øC
Reference reading = +24.5øC.
slope.
−47 øC/s.
slope.
+46 øC/s.
Thermocouple reading = −196øC
Reference reading = −195.8øC
Temperature (øC).
100
50
0
−50
−100
−150
−178
−196
−200
Time (seconds)

FIG 5

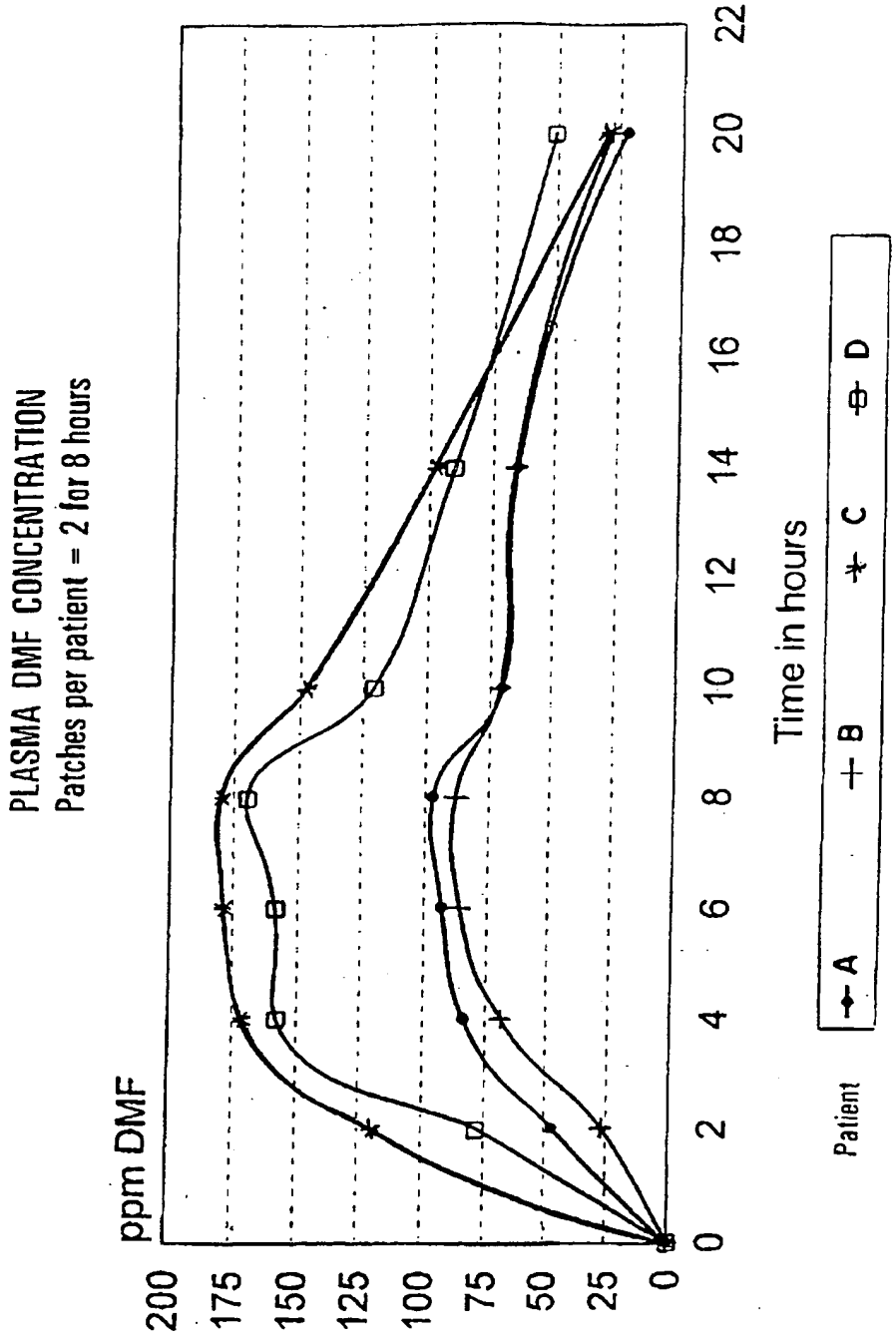

PLASMA DMF CONCENTRATION
Patches per patient = 2 for 8 hours
ppm DMF
200
175
150
125
100
75
50
25
0
0
2
4
6
8
10
12
14
16
18
20
22
Time in hours
Patient
A
B
C
D

FIG 6

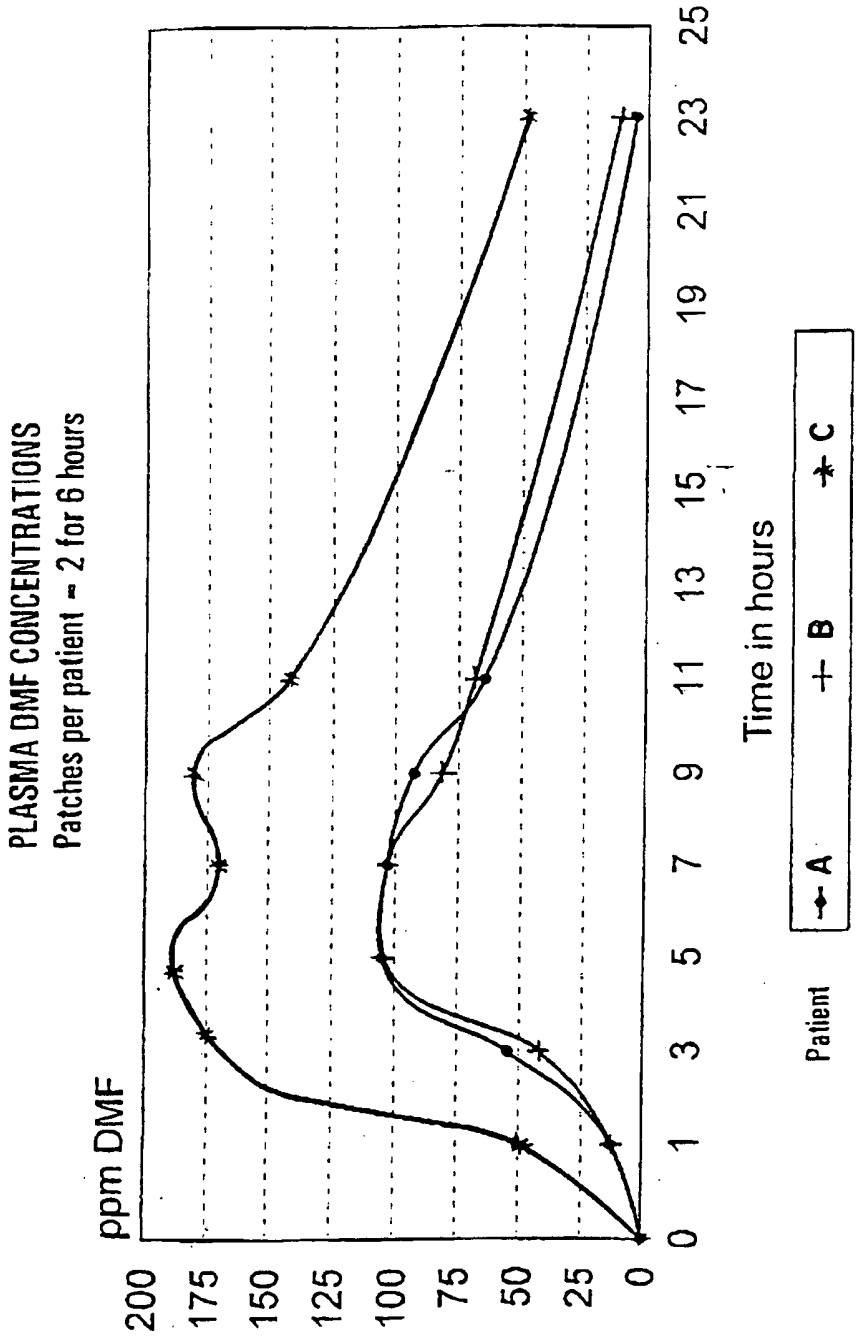

PLASMA DMF CONCENTRATIONS
Patches per patient = 2 for 6 hours
ppm DMF
200
175
150
125
100
75
50
25
0
0 1 3 5 7 9 11 13 15 17 19 21 23 25
Time in hours
Patient
A
B
C

FIG 7

FIG 8

FIG 9

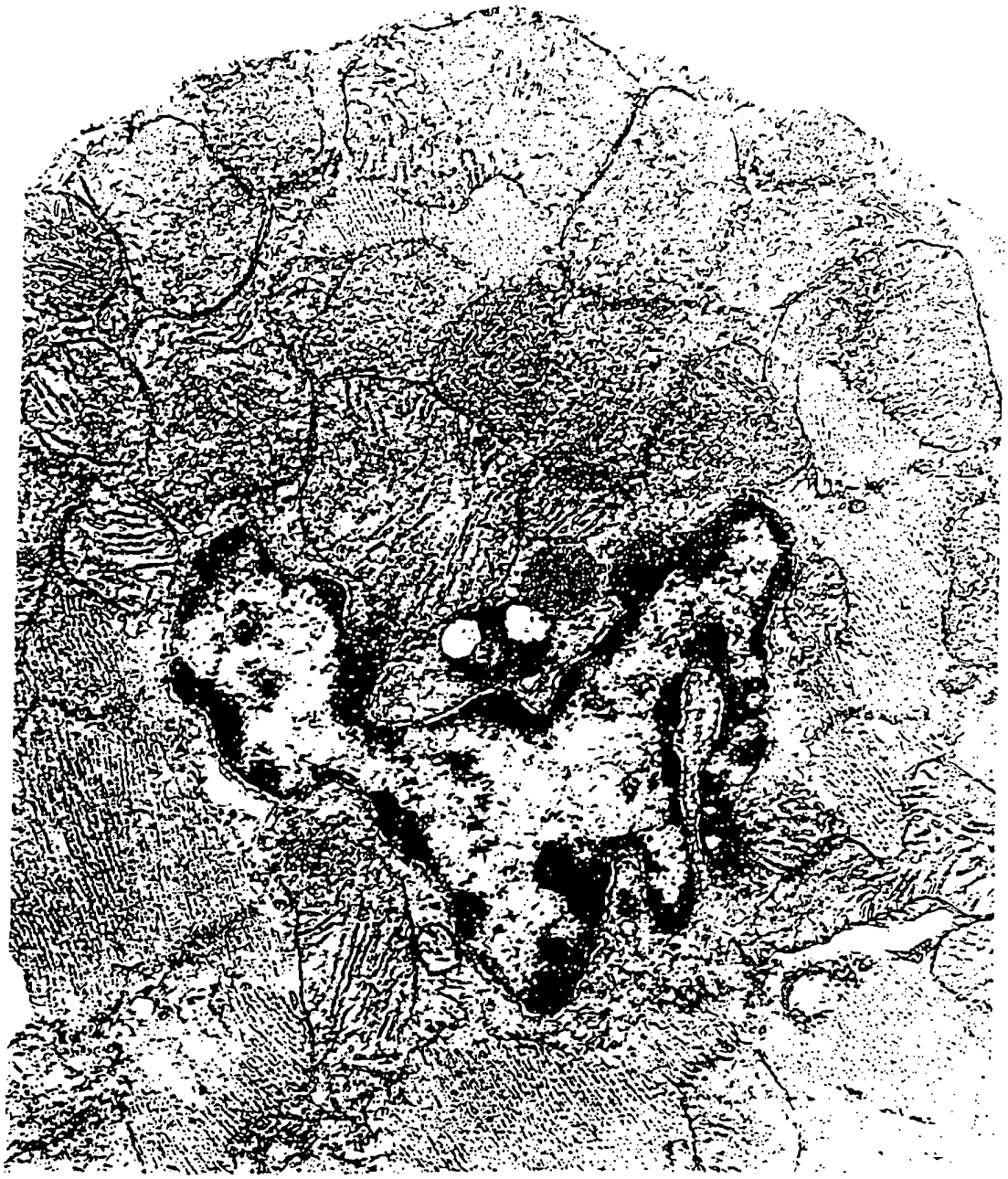


FIG 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19697

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A01N 37/18; A61K 31/16
US CL :514/627, 628, 629
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/627, 628, 629

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database CAPLUS on STN, WO 93/00808, American National Red Cross, 21 January 1993, Abstract No. 118:142966.	1-11, 20, 22, 24, 26-30, 38, 39, 41-43, 45-48, 50, 51
X	Database Medline on STN, Journal of Clinical Oncology 2(3), SPREMULLI et al., March 1984.	1-5, 12-17, 21, 23, 25, 31-34, 36, 40, 42, 44, 46, 49-51

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
22 MARCH 1997

Date of mailing of the international search report
14 MAY 1997

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Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19697

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 18, 19, 35, 37
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19697

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

MEDLINE, REGISTRY, CAPLUS: organ or heart or kidney# or liver# or conrea# or lung#, croprotect? or cryopreserv?, amides, retroviridae, measles, acne, AND the structure of the compound.
IFICDB (CLAIMS): amide fragments, organs(biology), livers, kidneys, hearts, viricides, cryogenics